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Comment on the Significance of Positive Carcinogenicity Studies Using Gavage as the Route of Exposure

by Frederica Perera,* Troyan Brennan,† and James R. Fouts‡

There is continuing controversy, extending into regulatory matters, over the significance to human health of positive results in carcinogenicity studies in animals using the gavage technique as the route of exposure. Our review of a nonrandom sample of 117 chemicals or chemical processes listed as known or reasonably anticipated to be carcinogenic in the National Toxicology Program's Third Annual Report on Carcinogens provides support for the validity of the gavage route in such studies. Twenty-three chemicals among the 117 substances and processes listed were positive by gavage. Twenty of these 23 chemicals were also appropriately studied by at least one other route of exposure. Thus, we were able to evaluate the extent to which positive gavage results were confirmed by another route of exposure in this sample. Nineteen (or 95%) of the twenty chemicals were positive for carcinogenicity by at least one other nongavage route in carcinogenicity bioassays. Moreover, in each of these 19 cases, positive carcinogenesis results were obtained by a nongavage route in the same species of animal where gavage administration led to the induction of cancer. All of the 23 gavage-positive chemicals induced tumors distal to the site of administration in at least one study, as did all 15 chemicals which were also positive by subcutaneous injection. We emphasize, however, the limited scope of our survey. We have not evaluated all chemicals that have tested positive by gavage and by at least one alternative route, nor have we assessed those chemicals found to be negative by the gavage route. Despite this limitation, our review suggests that, although gavage may not be the general method of choice for chemical administration, the results of studies wherein this route was employed are meaningful as a basis for assessing potential carcinogenic hazards.

Introduction

The scientific and regulatory communities generally agree that chemicals positive in properly conducted carcinogenesis studies should be regarded, for practical purposes, as likely to be carcinogenic in humans (*1,2*). However, positive results on a number of commercially important substances in animal studies wherein gavage was the route of administration have triggered a debate about the validity of those results (*3,4*). This has been especially true when vegetable oil was the vehicle. The debate centers around the possibility that results obtained by the gavage route may be misleading—especially that gavage may lead to an excess of false positive results as compared with other routes (*3*). This question is particularly relevant to the assays conducted in the National Toxicology Program (NTP) where, in the past, the gavage route was often used in carcinogenesis studies. This debate about the reliability of the gavage route was central in discussions about the cancellation of the pesticide ethylene dibromide (EDB) by the Environmental Protection Agency (EPA) (*5–7*). However, like two other gavage-positive chemicals of regulatory importance, 1,2-dibromo-3-chloropropane (*8*) and benzene (*9–11*), EDB has recently been demonstrated to be carcinogenic by the inhalation route as well.

In principle and where possible, carcinogenicity bioassays should employ a route relevant to anticipated human exposure (*4*), but this may not always be practicable. For example, in the case of water insoluble, volatile, or unstable compounds, or substances that are unpalatable to test animals, gavage may be the route of choice to ensure adequate and quantifiable dose and absorption of the test substance. The vehicle chosen may be oil (usually edible vegetable oil) to overcome problems such as hydrophobicity. For these practical reasons, gavage becomes the most convenient and accurate route of administration for many tests (*2*).

Concern has been voiced that the oil vehicle used in administering some chemicals by gavage may alter the rate of absorption, distribution, excretion, and metabolism of

*Columbia University School of Public Health, 60 Haven Avenue, B-109, New York, NY 10032.
†Massachusetts General Hospital, Boston, MA 02114.
‡National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

the test substance, or may affect hormonal status, cell division or other factors that modify tumorigenic responses (*12–14*). At the present time, there is only limited information regarding the ability of the gavage vehicle to modify the pharmacokinetics of test substances, and the significance of nutritional, physiological, and biochemical effects induced by various types of oil gavage is not well established. However, it should be pointed out that the vegetable oil used in most studies is identical to one of the common constituents of most human diets. There are also some reports of a tumor-promoting effect of oils used in administering chemicals by gavage (*4,14,15*) and of an overall elevated incidence of pancreatic acinar adenoma in male F344/N rats receiving corn oil by gavage in some studies (*16*). Yet there have been marked variations in the incidence of both these effects, and the most consistent effect in gavage studies using corn oil as the vehicle was a decrease in the incidence of leukemia (*17*).

It has also been argued that in most cases (except for ingested materials including drugs to be taken orally), the gavage route bears little relationship to anticipated human exposure to the toxic substance (*18*). However, many materials that are inhaled will end up in the stomach, often in large amounts. This is particularly true of materials suspended as particles in the air. Inhaled particles, deposited in the lung, can be transported up the respiratory tree and then swallowed. Thus, oral ingestion is a common route of human exposure (ultimately) to carcinogens in the environment or workplace.

Questions about the biological effects of gavage and vehicles used in administration are difficult to resolve because they are likely to be affected by factors such as animal age or weight, species and strain, the amount and type of vehicle (oil) used, the presence or absence of food in the stomach at the time of gavage, the time and frequency of the gavage, and the skill of the person administering the gavage (to minimize the stress or injury to the animal). Rates of absorption of oilborne materials from the site of administration greatly depend on all these factors, as well as the rate of stomach emptying, which is also affected by anxiety, light/dark cycles, etc. Adequate information on such factors is rarely available for gavage nor for any other route. (Further research is obviously needed on all these questions.)

However, carcinogenicity data exist that can be analyzed to compare the results of various routes of administration at least qualitatively. We have therefore reviewed a recent NTP summary of test data (*19*) to compare the results of gavage with other routes of administration. We examined the question: Does the use of gavage as the route of administration in the carcinogen bioassay frequently give positive data that are not confirmed when the same chemicals are tested by other routes of exposure?

Methods

Our source of data about carcinogenesis studies was the NTP Third Annual Report on Carcinogens, a listing of 117 chemicals or chemical processes known or reasonably anticipated to be carcinogens (*19*). Of these, 23 chemicals were positive in experiments using gavage as the route of exposure. Each of the 23 chemicals was reviewed to see if, according to the NTP or the International Agency for Research on Cancer (IARC) data base, tumors had been induced by the same chemical via other routes of administration, and in the same or a different animal species (Table 1). We have relied on the conclusions of the NTP and IARC as to whether the chemical was carcinogenic in the individual studies and have not performed additional statistical analysis of the significance of results characterized by these agencies as positive.

Table 1 compares the results of gavage studies to other routes of exposure. Several, but not all, conceivable alternative routes are compared. Often only one study was cited for each route, although in many cases there are reports of more than one positive study by the same route. To avoid possible false positive results in studies using the SC route of administration, positive results where the tumors produced were distal to the site of injection were distinguished from those where the only tumors seen arose at the injection site (*20*).

Results and Discussion

Of the 23 chemicals or processes listed in the NTP Third Annual Report on Carcinogens and administered by the gavage route (19 of which are listed in Table 1), only 4 [chloroform (*21*), dichloroethane (*22*), polybrominated biphenyls (*23*), and selenium sulfide (*24*)] have not been reported also as positive in at least one other study wherein a different route of administration was used. However, of these 4, only selenium sulfide has been adequately studied by an alternative (in this case dermal) route. We found no reports of studies of PBBs where nongavage routes were employed. Although negative results were obtained when chloroform and dichloroethane were injected IP in rats, these authors and the IARC consider this test system limited and view the negative results of such studies as insufficient evidence of noncarcinogenicity (*25*). Thus, selenium sulfide is the only chemical studied by at least one appropriate alternative route whose positive results by the gavage route are not confirmed (*24,26*). In addition, every chemical listed in Table 1 was found to be positive in a study using a nongavage route in the same species of animal that gave positive results when the chemical was administered by gavage.

Table 1 shows that, in at least one study, each chemical induced tumors distal to the site of application (stomach). This was also true for the 15 substances in this data set studied by the SC route. In at least one SC experiment, all 15 chemicals caused tumors at sites other than (sometimes in addition to) the injection site: 4-amino-biphenyl, benzidine, benzo[a]pyrene, carbon tetrachloride, cycasin, dibenz[a,h]anthracene, 7H-dibenzo[c,g]carbazole, diethylstilbestrol, dimethylbenzidine, 2-naphthylamine, *N*-nitrosodibutylamine, *N*-nitrosodiethylamine, *N*-nitrosodimethylamine, *N*-nitroso-*N*-methylurea, and urethane. Thus, the

Table 1. Chemicals positive by gavage and other routes.[a]

Chemical	Species	Route of administration	Tumor site(s)	Reference
Acrylonitrile	Rat	Gavage	Forestomach, breast	(27)
	Rat	Inhalation	Zymbal gland, breast, forestomach, brain, skin	(27)
	Rat	Drinking water	Brain, Zymbal gland, stomach	(28)
4-Aminobiphenyl	Mouse	Gavage	Bladder	(29)
	Mouse	Drinking water	Bladder, angiosarcoma, heptocellular neoplasms	(30)
	Rat	Subcutaneous	Large intestine, breast, uterus	(31)
Benzidine	Rat	Gavage	Breast	(32)
	Rat	Subcutaneous	Liver, Zymbal gland, local (injection site)	(33)
	Rat	Intraperitoneal	Mammary, Zymbal gland	(33)
	Hamster	Oral	Liver	(34)
Benzo[a]pyrene	Hamster	Gavage	Forestomach, trachea	(35)
	Mouse	Gavage	Mammary	(36)
	Rat	Intrabronchial implant	Lung, bronchus	(37)
	Rat	Intratracheal implant	Trachea	(37)
	Mouse	Diet	Stomach, lung, leukemias	(38)
	Mouse	Subcutaneous	Lung, breast	(39)
	Mouse	Intraperitoneal	Lung, lymphoma	(39)
	Mouse	Subcutaneous	Local (injection site)	(40,41)
	Hamster	Intrabronchial implant	Bronchus	(37)
	Hamster	Mouth/spray	Skin, trachea, stomach	(35)
Carbon tetrachloride	Mouse	Gavage	Liver	(21,42,43)
	Mouse	Oral	Liver	(44)
	Rat	Inhalation	Liver	(44)
	Rat	Subcutaneous	Liver, thryoid, spleen	(35,43)
Cycasin	Mouse	Gavage	Liver, kidney	(46)
	Mouse	Subcutaneous	Liver, lung	(46,47)
	Mouse	Topical	Liver, kidney	(48)
	Rat	Diet	Liver, kidney, lung, intestine	(49)
Dibenz[a,h]anthracene	Mouse	Gavage	Breast, forestomach, intestine, lung	(36,50)
	Mouse	Subcutaneous	Local (injection site), skin, lung	(41)
	Mouse	Topical	Skin	(51)
	Rat	Inhalation	Lung	(52)
7H-dibenzo[c,g]carbazole	Mouse	Gavage	Forestomach, liver, lung	(53
	Mouse	Topical	Skin	(54,55)
	Mouse	Subcutaneous	Local (injection site), liver	(54)
	Mouse	Bladder, implant	Bladder	(54)
	Hamster	Intratracheal instillation	Lung, bronchus, trachea, forestomach	(57)
	Rat	Subcutaneous	Skin	(55)
Diethylstilbestrol	Mouse	Gavage	Breast	(58)
	Mouse	Diet	Breast	(59)
	Rat	Subcutaneous	Pituitary	(60)
Dimethylbenzidine	Rat	Gavage	Breast	(32)
	Rat	Subcutaneous (pellets)	Skin, Zymbal and preputial glands, breast, liver, intestine, forestomach	(61)
Hydrazine	Mouse	Gavage	Liver, lung	(62)
	Rat	Gavage	Liver, lung	(62)
	Mouse	Oral	Liver, lung	(63)
2-Naphthylamine	Mouse	Gavage	Liver	(56)
	Mouse	Diet	Liver	(56,64)
	Mouse	Subcutaneous	Liver, local (injection site)	(64)
	Rat	Diet	Bladder	(56)
	Hamster	Diet	Bladder, liver	(34)
	Dog	Diet	Bladder	(64)
N-Nitrosodibutylamine	Hamster	Gavage	Bladder, trachea, lung, forestomach	(65)
	Hamster	Subcutaneous	Bladder, trachea, lung	(65)
	Mouse	Diet	Forestomach, lung, liver	(66)
	Mouse	Subcutaneous	Liver	(67)
N-nitrosodiethylamine	Hamster	Gavage	Lung, trachea	(68)
	Hamster	Oral	Stomach, esophagus	(69)
	Hamster	Topical	Nose	(69)
	Hamster	Inhalation	Lung, trachea, bronchi	(69)
	Hamster	Subcutaneous	Nose, lung, trachea, liver, stomach	(69)

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Table 1. (Continued)

Chemical	Species	Route of administration	Tumor site(s)	Reference
	Hamster	Intraperitoneal	Nose, trachea, liver	(69)
	Rat	Inhalation	Liver	(70)
	Rat	Oral	Liver, esophagus, nose	(67,71,72)
	Rat	Intravenous	Liver, mouth, pharynx, esophagus	(68)
	Mouse	Subcutaneous	Liver, lung, nose	(72)
	Mouse	Topical	Nose	(73)
N-Nitrosodimethylamine	Hamster	Gavage	Liver, stomach	(75)
	Hamster	Subcutaneous	Liver, lung, nose	(76)
	Rat	Inhalation	Nose, pituitary, kidney	(72)
	Rat	Oral	Liver, kidney	(72)
	Rat	Diet	Liver, kidney, lung	(77,78)
	Mouse	Subcutaneous	Lung, breast, local (site of injection)	(79)
N-Nitroso-*N*-methylurea	Rat	Gavage	Kidney, stomach, small intestine, large intestine, skin, jaw	(80)
	Rat	Oral	Kidney, brain	(81)
	Rat	Topical	Skin	(82)
	Hamster	Intratracheal instillation	All parts of pulmonary tree, esophagus, forestomach, skin	(83)
	Hamster	Topical	Skin	(82)
	Mouse	Subcutaneous	Lymphoma, lymphosarcoma	(84)
	Mouse	Topical	Skin	(82)
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	Rat	Gavage	Thyroid, liver	(85)
	Mouse	Gavage	Thyroid, liver	(85)
	Mouse	Topical	Skin (integumentary system)	(86)
	Rat	Diet	Liver, lung, nose, hard palate, adrenal cortex	(85)
Urethane	Mouse	Gavage	Liver, lung, forestomach, leukemia, reticulum cell	(87)
	Mouse	Inhalation	Lung	(88)
	Mouse	Oral	Lymphoma, lung, skin	(89)
	Mouse	Subcutaneous	Lymphoma, lung	(37)
	Mouse	Topical	Skin, lung, liver	(90)
Vinyl chloride	Rat	Gavage	Zymbal gland, kidney, liver, skin, brain, breast	(91)
	Rat	Interperitoneal	Kidney, subcutaneous	(91)
	Rat	Inhalation	Zymbal gland, kidney, liver, skin	(91,92)
	Hamster	Inhalation	Liver, skin, forestomach, lymphoma	(91,92)

possible objection that studies of carcinogenesis wherein the SC route of administration was used might be flawed by the finding of tumors only at the site of injection does not seem to apply to our analysis. The SC route was therefore used to validate the results of studies using the gavage route. However, we did not exclude from the table carcinogenic responses at the site of administration (whether this be in the skin after SC injection or in the forestomach after gavage) since these are also informative. There can be many reasons for such results, among them the longer residence time for the chemical substance at this site. Carcinogenicity is a function of exposure (both dose and time), which varies so widely from one situation to another depending on animal age, sex, strain of the animal, site, vehicle, and dose that all positive results should be carefully considered. Unless it can be shown that factors that cause tumors at the site of administration do not operate distally, it seems reasonable to consider administration site tumors as biologically relevant. In fact, intermittent, high concentrations of carcinogenic chemicals at sites of entry are frequent in humans.

As mentioned earlier, in an analysis of the NTP historical control data base and of nearly 300 carcinogenesis studies carried out by the National Cancer Institute and NTP (*17*), some concern was raised about the use of an oil vehicle in gavage since in male F344/N control rats receiving corn oil by gavage there was an increase in pancreatic acinar cell adenoma and a decrease in leukemia as compared with untreated controls. Interpretation of these results is difficult. First, effects were variable from one study to another, and no other tumor incidences seemed to be affected by the gavage. Second, in no gavage study using corn oil as the vehicle was the increased incidence in pancreatic acinar cell tumors the sole evidence of carcinogenicity of any test chemical (*17*).

In summary, the present review seeks to answer a simple question: Is the gavage route giving a high rate of false positive results in a sample of chemicals tested? This was not the case in the survey we conducted. Nor would we anticipate a different result if additional chemicals from the subsequent NTP Annual Reports were included in a similar survey, as they would have been selected according to the same criteria as in the Third Annual Report. However, we emphasize that this survey is a first step in evaluation of the question at hand. We have not performed a global evaluation of all gavage-positive chemicals that have been adequately tested by at least one additional route. Nor have we assessed gavage-negative chemicals. Both were outside the scope of this report. In particular, evaluation of negative studies would have re-

quired a critical review of the design and power of each study to exclude possible false negatives. Despite these limitations, the data suggest that gavage studies can provide valuable evidence that might be used in assessing the potential of a chemical to be a human carcinogen and that the results of carcinogenesis studies using the gavage route of administration should not be discounted. In this sample of chemicals, in every case but one, positive results by gavage were confirmed by assays using other appropriate routes of administration.

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